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10/506,958	05/02/2005	Helen Braven	ATLAS 8095 US	8800

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EXAMINER	
POHNERT, STEVEN C	
ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
07/14/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/506,958	BRAVEN ET AL.	
	Examiner	Art Unit	
	STEVEN C. POHNERT	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 April 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,11-25,43-45,91-105 and 109-133* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9,11-25,43-45,91-105 and 109-133* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *07 September 2004* is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/21/2009 has been entered.

Formal matters and Claim Status

The instant action is in response to the claim amendments of 4/21/2010 and the RCE filed 10/21/2009.

Claims 1-9, 11-45 and 91-105 and 109-133 are pending and being examined.

Claims 1 and 109 have been amended.

The previous rejection based on CMS and Calzone has been withdrawn in view of the amendment.

It is noted that the interview summary of 4/21/2010 is not complete as the instant response lacks an acknowledgment of the interview. In promoting compact prosecution of the instant application, the examiner is examining the instant application. The next response by applicant must complete the record of the interview which took place on 4/21/2010, or it may be held as not fully responsive.

Claim Rejections - 35 USC § 112

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claims 43-45, 99-105, 119-123 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 43-45 recites the limitation "the electrochemically determined information " and depend from claim 1. There is insufficient antecedent basis for this limitation in the claim as it is not previously recited in the claim or the claim from which it depends. This rejection may be overcome by amending the claim to recite, "the electrochemical activity of the electrochemical marker."

4. Claims 99-101 recites the limitation "the electrochemical step" and depend from claim 1. There is insufficient antecedent basis for this limitation in the claim or claim 1 from which they depend. This rejection can easily be overcome by amending the claim to recite, "determining the activity of the electrochemical marker."

5. Claim 102 recites the limitation "the electrochemical technique" in the first line. There is insufficient antecedent basis for this limitation in the claim. This rejection cha be over come by amending the claim to recite, "Determining the activity of the electrochemical marker." Claims 103-105 are rejected as they depend from claim 102.

6. Claims 119-123 recites the limitation "the electrochemically determined information " and depend from claim 109. There is insufficient antecedent basis for this limitation in the claim as it is not previously recited in the claim or the claim from which it depends. This rejection may be overcome by amending the claim to recite, "the electrochemical activity of the electrochemical marker."

7. Claims 127-129 recites the limitation "the electrochemical step" and depend from claim 109. There is insufficient antecedent basis for this limitation in the claim or claim 109 from which they depend. This rejection can easily be overcome by amending the claim to recite, "determining the activity of the electrochemical marker."

8. Claim 130 recites the limitation "the electrochemical technique" in the first line. There is insufficient antecedent basis for this limitation in the claim. This rejection cha be over come by amending the claim to recite, "Determining the activity of the electrochemical marker." Claims 131-133 are rejected as they depend from claim 130.

Claim Rejections - 35 USC § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

11. Claims 1-3, 11-18, 20-25, 91-92, 99 and 101 are rejected under 35 U.S.C. 103(a) as being unpatentable over Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001) in view Kayyem et al (US patent 6,096, 273 issued August 1, 2000) and Meade et al (WO 95/15971, published June 15, 1995).

Clinical Micro Sensors is here after referred to as CMS.

With regards to claim 1, CMS et al teaches a method of detecting a nucleic acid by use of a probe labeled with electron transfer moieties (10 and 12) (electrochemically active marker) linked by a scissile linker (11) which comprises (1) at the top of figure 32. CMS further teaches the two probe linker complex (1) is provided conditions in which it can hybridize to the target sequence (120) forming complex (6), the linker is then cleaved (or degraded). CMS further teaches the detection (electrochemically determining information) of both probes 10 and 11 by electron transfer (electrochemically), thus detecting the presence of the target nucleotide (bottom of figure 32). CMS further teaches the detection of the non-degraded probe.

CMS further teaches that the electronic transport is based on the size of the nucleic acid (104). CMS teaches non-specifically bound label probes/ ETM have differences in impedance and non-specific material is washed away (108). CMS teaches that faradic impedance can be measured will depend on the distance of an ETM from the electrode, and thus the length of the nucleic acid (112).

With regards to claim 2, the method of CMS allows detection of target sequence 120, by electrochemical detection of probes 10 and 12 (see figure 32).

With regards to claim 3, CMS teaches the method allows for the quantifiable detection of the rate of generation of cleaved fragments or the amount of final products (see page 34, lines 8-18). As CMS teaches the use of specified amounts of probes, CMS thus inherently teaches the quantifiable detection of relative portions of degraded and non-degraded probes.

With regards to claims 11 and 12, CMS teaches the use of Invader™ technology as a preferred embodiment. CMS teaches the use of an "invader primer and a signaling primer that has an overlapping sequence(see page 42, lines 8-11). CMS further teaches that invader technology is based on structure specific polymerases that cleave nucleic acids in a site-specific manner (see page 42, lines 1-3). With regards to claim 12, CMS teaches the use of 5' thermostable nucleases (see page 42, lime 16).

With regard to claim 13, CMS further teaches that invader technology is based on structure specific polymerases that cleave nucleic acids in a site-specific manner (see page 42, lines 1-3).

With regards to claims 14 and 15, CMS further teaches this polymerase/nuclease can be from Taq (see page 42, line 16).

With regards to claim 16, CMS teaches a new primer binds after cleavage (see page 42, line 19). CMS thus teaches a solution comprises a primer pair suitable for extension.

With regards to claim 17, CMS teaches PCR amplification using Taq polymerase by cycling in the preferred embodiment (see page 21, lines 16-20).

With regards to claim 18, CMS teaches the use of invader technology using two probes. Invader technology is based on hybridization of first oligonucleotide and a second oligonucleotide to a target sequence, with a non-complementary overlap that is cleaved by a nuclease (see page 42, lines 1-25). CMS further the ETM tagged tail is released in the cleavage by a nuclease that specifically recognizes the structure of the 2 probe target complex, the cleavage releasing the tail with an ETM tag (see page 42, lines 1-5 and lines 21-25). This cleavage shortens the oligonucleotide to which the ETM is attached.

With regards to claim 20, CMS teaches the detection of mutations, which are nucleic acid polymorphisms (see page 113, line 12).

With regards to claim 21, CMS teaches the detection of BRCA1, P53, and APOE4 for the presymptomatic screening of patients. CMS thus teaches detection of allelic polymorphisms (see page 113, line 12).

With regards to claim 22, CMS teaches the probe array for use in sequencing by hybridization which would determine single nucleotide polymorphisms (see page 113, line 33).

With regards to claim 23, CMS teaches it method allows detection of 10^6 molecules (see page 114, line 35). CMS thus teaches the quantifiable detection of nucleic acid species.

With regards to claim 24, CMS teaches its method can be used for the detection of mRNA (see page 114, lines 18).

With regards to claim 25, CMS teaches the use of software directed microprocessor for the detection of electrochemical active species (figure 20A).

With regards to claim 91, CMS teaches in figure 32 the use of two oligonucleotide probes 10 and 12 with two different EMT probes 135 and 13.

With regards to claim 92, CMS teaches in figure 20 a-o, that two or labels can be distinguished by peaks on their voltametric traces.

With regards to claim 99, CMS teaches the use of voltammetry methods for detection (see page 105, line 10).

With regards to claim 100, CMS teaches the use of amperommetry for detection (see page 105, line 10).

With regards to claim 101, CMS teaches the use of differential pulse voltametery (see page 106, lines 25-26).

CMS does not specifically teach that the electrochemical characteristics are different depending on whether the electrochemically active label is attached to a nucleotide, is incorporated into an oligonucleotide or the length of the oligonucleotide.

However, Kayyem teaches the detection of nucleic acid by use of metallocene for electron transport along the stacked π orbital of double stranded DNA. Kayyem teaches the electron transfer moieties taught by Meade allow for this detection (column 4).

Meade teaches, "This differential in the rate of electron transfer forms the basic of a utility of the present invention for use as probes. In the system of the present invention, where electron transfer moieties are covalently bound to the backbone of a

nucleic acid, the electrons putatively travel via the π-orbitals of the stacked base pairs of the double stranded nucleic acid. The electron transfer rate is dependent on several factors, including the distance between the electron donor-acceptor pair, the free energy (ΔG) of the reaction, the reorganization energy (λ) , the contribution of the intervening medium, the orientation and electronic coupling of the donor and acceptor pair, and the hydrogen bonding between the base. The latter confers a dependence on the actual nucleic acid sequence, since A-T pairs contain one less hydrogen bond than C-G pairs. However, this sequence dependence is overshadowed by the determination that there is a measurable difference between the rate of electron transfer within a DNA base-pair matrix, and the rate through the ribose-phosphate backbone, the solvent or other electron tunnels. This rate differential is thought to be at least several orders of magnitude, and may be as high as four orders of magnitude greater through the stacked nucleotide bases as compared to other electron transfer pathways. Thus the presence of double stranded nucleic acids, for example in gene probe assays, can be determined by comparing the rate of electron transfer for the unhybridized probe with the rate for hybridized probes.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made that the ETM as exemplified by metallocene taught by CMS have a different electrochemical characteristics when attached to a base in an oligonucleotide as the art of record recognizes the ETM attached to an oligonucleotide that is hybridized allows flow through the π orbital electrons which is not possible if the ETM is not attached to a base. Further it is obvious over the prior art that the

electrochemical characteristics are dependent on the distance of the ETM from the electrode and thus the length of the nucleic acid. The artisan would have a reasonable expectation of success as the art demonstrates the claimed limitations are properties of the metallocene taught by CMS.

Response to Arguments

This is a new grounds of rejection. Thus the response has no specific arguments to the instant rejection.

However, the response asserts the teachings of CMS do not teach the electrochemical characteristics depending whether the marker is attached to a nucleotide, incorporated into an oligonucleotide, and the length of oligonucleotide. These arguments have been thoroughly reviewed but are not considered persuasive as the combined teachings of CMS, Kayyem and mead demonstrate these limitations are obvious. Further it is noted teachings of the art of record encompass the metallocene labels of the instant specification, thus these alleged differences appear to be inherent properties of the metallocene labels of nucleic acids absent secondary considerations. Thus the claims are obvious over the teachings of CMS, Kayyem and Mead.

12. Claim 93 is rejected under 35 U.S.C. 103(a) as being unpatentable over Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001), Kayyem et al (US patent 6,096, 273 issued August 1, 2000) and Meade et al (WO 95/15971, published June 15, 1995) as applied to claims 1-3, 11-18, 20-25, 91-92, 99 and 101 above, and further in view of Nikiforov et al (US Patent issued May 21, 1996).

The teachings of CMS, Kayyem and Meade are set forth above.

CMS, Kayyem and Meade do not teach the use T7 exonuclease.

However, Nikiforov et al teaches the use of T7 exonuclease in primer extension assays to generate single stranded nucleic acids (see abstract). Nikiforov et al teaches that T7 exonuclease has the advantage over other nucleases that it has maximal activity in buffers suitable for DNA polymerase activity.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to improve the method of CMS, Kayyem and Meade nucleic acid detection method by use of the T7 exonuclease taught by Nikiforov, because Nikiforov teaches T7 exonuclease can be used in the same buffer that amplification. The artisan would have a reasonable expectation of using known molecular biology enzyme in a known assay.

13. Claims 102-105 are rejected under 35 U.S.C. 103(a) as being unpatentable over Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001), Kayyem et al (US patent 6,096, 273 issued August 1, 2000) and Meade et al (WO 95/15971, published June 15, 1995) as applied to claims 1-3, 11-18, 20-25, 91-92, 99 and 101 above in view of Heller et al (US Patent 5,605,662, filed Issued February 25, 1997).

Clinical Micro Sensors is here after referred to as CMS.

CMS, Kayyem and Meade do not teach the use of electrochemical technique utilizing selectively one or more electrodes functionally surrounded by permeable membrane that is permeable on the basis of charge, size, or hydorphobicity.

However, Heller et al teaches the use of permeation layers covering electrodes that allow solvent movement, while allowing exclusion based on size and charge (see

column 11, lines 3-23; column 13, lines 30-55). Heller teaches the use of charge in the permeability layer he also inherently teaches the use of hydrophilic layers, as charge molecules are hydorphillic. Heller teaches the permeation layer functionally surrounding the electrode inhibits large proteins in the sample from binding the electrode, thus allowing the use of DC current (see column 11, lines 17-35). If the large proteins bound to the electrode, the large proteins would act as insulators, and cause a short circuit.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of CMS, Kayyem and Meade to include the use of a electrode with a selectively permeable membrane (permeation layer) of Heller, because Heller teaches the permeation layer allows the use of direct current without the insulating effects of large proteins binding to the electrode. The use of Heller's permeation layer would thus result in more accurate and sensitive assays. The artisan would have a reasonable expectation of success combining known molecular biology methods.

14. Claim 19 is rejected under 35 U.S.C. 103(a) as being unpatentable over Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001), Kayyem et al (US patent 6,096, 273 issued August 1, 2000) and Meade et al (WO 95/15971, published June 15, 1995) as applied to claims 1-3, 11-18, 20-25, 91-92, 99 and 101 view of Hall et al (US Patent 5,994,069 issued November 30, 1999).

Clinical Micro Sensors is here after referred to as CMS.

The teachings of CMS, Kayyem and Meade are set forth above.

CMS, Kayyem and Meade do not teach the use of a second recognition cassette that is labeled for detection of the cleavage reaction of a first partially hybridized complex.

However, Hall et al teaches a method of signal amplification using an invader probe, an unlabeled 1st probe, and a labeled second probe (see figure 96, and column 71 lines 45-52). Hall teaches the invader probe binds a first target sequence, while the 1st probe partially hybridizes the target and the unhybridized 5' tail of the 1st probe is released. The 5' tail of the 1st probe than hybridize a second target sequence, causing the 5' tail of the 2nd labeled probe to only partially hybridize to the second target sequence. Hall teaches the labeled 5' end of the second sequence is thus cleaved, released, and detected. The 2nd labeled probe and 2nd target are a recognition cassette.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improvement of the ETM invader method of CMS, Kayyem and Meade by use of Halls 2nd labeled probe and target. The ordinary artisan would be motivated to improve CMS, Kayyem and Meade method by use of Hall's 2nd labeled probe and target, because Hall teaches it amplifies the signal, resulting in a more sensitive assay. The combined teachings of CMS, Kayyem and Meade and Hall would result in a more sensitive electrochemical invader assay, than that taught by CMS.

15. Claims 1-9, 11-18, 20-25, 43-45, 91-101, 109-116, 119-129 are rejected under 35 U.S.C. 103(a) as being unpatentable over Calzone et al (Methods in Enzymology

(1987) volume 152, pages 611-632) in view of Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001), Kayyem et al (US patent 6,096, 273 issued August 1, 2000) and Meade et al (WO 95/15971, published June 15, 1995).

Clinical Micro Sensors is here after referred to as CMS.

With regards to claim 1 and 109, Calzone teaches a method of mapping gene transcripts by nuclease protection assay to determine the size and length of the nucleic acid (see title, figure 1). Figure 1 teaches the labeled probes are hybridized and then digested by S1 nuclease or Exonuclease. Calzone teaches the digest complex is run on a gel allowing for determination of size and characteristics of the probe and thus the nucleic acid.

With regards to claim 2 and 112, the presence of degraded probe of Calzone teaches the presence of the nucleic acid.

With regards to claim 3, 94 and 113, Calzone teaches in figure 2 the relative portions of degraded and non-degraded probes as the 520 base primary transcript and the 75 base mature mRNA.

With regards to claim 4, Calzone teaches the use of nucleases that selectively digest single strand nucleic acid (see figure 1).

With regards to claim 5, 97Calzone teaches the use of mung bean endonuclease (see page 612, 1st paragraph).

With regards to claim 6, 96 Calzone teaches the use of RNase A to digest excess RNA (page 620, 1st paragraph).

With regards to claims 7, 8, 98 Calzone teaches the use of S1, which is a DNAse (see figure 1).

With regards to claim 9, 95 Calzone teaches the use of exonuclease VII (see figure 1).

With regards to claim 11, Calzone teaches the use of nucleases that selectively digest free strands of double stranded hybridized duplexes. Thus the selective degradation of non-hybridized segments of the hybridized nucleic acid is selective digesting at least one strand.

With regards to claim 12 and 110-111, Calzone teaches that exonuclease VII hydrolyzes single stranded DNA in the 5' direction (page 612, 1st paragraph). Calzone thus teaches an enzyme that is a 5' nuclease.

Calzone does not teach an electrochemically active marker. Calzone does not specifically teach that the electrochemical characteristics are different depending on whether the electrochemically active label is attached to a nucleotide, is incorporated into an oligonucleotide or the length of the oligonucleotide. Calzone does not teach a 5' nuclease that is a polymerase (claim 13). Calzone does not teach a thermostable 5' nuclease/DNA polymerase (claims 14-15). Calzone does not teach detection of mutations, polymorphisms, or quantitation of nucleic acids (claims 20-24, 43, 119-123). Calzone does not teach software for use in the method of detecting of pathogens (claims 25 and 44-45). Calzone does not teach the use of 2 probes with different electrochemical labels, or detection by voltametery, amperommetery, or differential pulse voltamtery (claims 91, 92, 99-101, 125-127).

However, Clinical Micro Sensors (CMS) teaches the use of electrochemical detection moieties (CMS calls ETMs) to as labels to detect nucleic acid sequences (see abstract). CMS further that ETMs allows amplification of signal resulting in sensitive assays (see page 54, lines 7-50). CMS teaches, "Thus, the present invention provides for extremely specific and sensitive probes, which may, in some embodiments, detect target sequences without removal of unhybridized probe. This will be useful in the generation of automated gene probe assays" (page 114). CMS teaches these labels and probes allow for automation (page 114).

CMS further teaches that the electronic transport is based on the size of the nucleic acid (104). CMS teaches non-specifically bound label probes/ ETM have differences in impedance and non-specific material is washed away (108). CMS teaches that faradic impedance can be measured will depend on the distance of an ETM from the electrode, and thus the length of the nucleic acid (112).

With regards to claims 11 and 12, CMS teaches the use of Invader^{tm} technology as a preferred embodiment. CMS teaches the use of an "invader primer and a signaling primer that has an overlapping sequence(see page 42, lines 8-11). CMS further teaches that invader technology is based on structure specific polymerases that cleave nucleic acids in a site-specific manner (see page 42, lines 1-3). With regards to claim 12, CMS teaches the use of 5' thermostable nucleases (see page 42, lime 16).

With regard to claim 13, CMS further teaches that invader technology is based on structure specific polymerases that cleave nucleic acids in a site-specific manner (see page 42, lines 1-3).

With regards to claims 14, 15, and 114-115, CMS further teaches this polymerase/nuclease can be from Taq (see page 42, line 16).

With regards to claims 16, and 124, CMS teaches a new primer binds after cleavage (see page 42, line 19). CMS thus teaches a solution comprises a primer pair suitable for extension.

With regards to claim 17, 115, 116 CMS teaches PCR amplification using Taq polymerase by cycling in the preferred embodiment (see page 21, lines 16-20).

With regards to claim 18, CMS teaches the use of invader technology using two probes. Invader technology is based on hybridization of first oligonucleotide and a second oligonucleotide to a target sequence, with a non-complementary overlap that is cleaved by a nuclease (see page 42, lines 1-25). CMS further the ETM tagged tail is released in the cleavage by a nuclease that specifically recognizes the structure of the 2 probe target complex, the cleavage releasing the tail with an ETM tag (see page 42, lines 1-5 and lines 21-25). This cleavage shortens the oligonucleotide to which the ETM is attached.

With regards to claims 20, 119, and 121 CMS teaches the detection of mutations, which are nucleic acid polymorphisms (see page 113, line 12).

With regards to claims 21, 43, and 120, CMS teaches the detection of BRCA1, P53, APOE4 for the presymptomatic screening of patients. CMS thus teaches detection of allelic polymorphisms (see page 113, line 12).

With regards to claim 22, CMS teaches the probe array for use in sequencing by hybridization which would determine single nucleotide polymorphisms (see page 113, line 33).

With regards to claim 23, 122, CMS teaches it method allows detection of 10^6 molecules (see page 114, line 35). CMS thus teaches the quantifiable detection of nucleic acid species.

With regards to claim 24, 123, CMS teaches its method can be used for the detection of mRNA (see page 114, lines 18).

With regards to claim 25, CMS teaches the use of software directed microprocessor for the detection of electrochemical active species (figure 20A).

With regards to claims 44 and 45, CMS teaches the detection of pathogens in a sample. CMS teaches, "The detection of specific nucleic acids is an important tool for diagnostic medicine and molecular biology research. Gene probe assays currently play roles in identifying infectious organisms such as bacteria and viruses, in probing the expression of normal genes and identifying mutant genes such as oncogenes, in typing tissue for compatibility preceding tissue transplantation, in matching tissue or blood samples for forensic medicine, and for exploring homology among genes from different species" (page 2, page 113). CMS thus teaches methods of detecting pathogens and therapy response (compatibility testing prior to transplantation).

With regards to claims 91 and 125 CMS teaches in figure 32 the use of two oligonucleotide probes 10 and 12 with two different EMT probes 135 and 13.

With regards to claims 92, 126, and 127, CMS teaches in figure 20 a-o, that two or labels can be distinguished by peaks on their voltametric traces.

With regards to claim 99, CMS teaches the use of voltammetry methods for detection (see page 105, line 10).

With regards to claims 100 and 128 CMS teaches the use of amperommetry for detection (see page 105, line 10).

With regards to claims 101 and 129 CMS teaches the use of differential pulse voltametery (see page 106, lines 25-26).

Kayyem teaches the detection of nucleic acid by use of metallocene for electron transport along the stacked π orbital of double stranded DNA. Kayyem teaches the electron transfer moieties taught by Meade allow for this detection (column 4).

Meade teaches, "This differential in the rate of electron transfer forms the basic of a utility of the present invention for use as probes. In the system of the present invention, where electron transfer moieties are covalently bound to the backbone of a nucleic acid, the electrons putatively travel via the π-orbitals of the stacked base pairs of the double stranded nucleic acid. The electron transfer rate is dependent on several factors, including the distance between the electron donor-acceptor pair, the free energy (ΔG) of the reaction, the reorganization energy (λ) , the contribution of the intervening medium, the orientation and electronic coupling of the donor and acceptor pair, and the hydrogen bonding between the base. The latter confers a dependence on the actual nucleic acid sequence, since A-T pairs contain one less hydrogen bond than C-G pairs. However, this sequence dependence is overshadowed by the determination that there

is a measurable difference between the rate of electron transfer within a DNA base-pair matrix, and the rate through the ribose-phosphate backbone, the solvent or other electron tunnels. This rate differential is thought to be at least several orders of magnitude, and may be as high as four orders of magnitude greater through the stacked nucleotide bases as compared to other electron transfer pathways. Thus the presence of double stranded nucleic acids, for example in gene probe assays, can be determined by comparing the rate of electron transfer for the unhybridized probe with the rate for hybridized probes.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of Calzone by use of the ETM labels and nucleases and methods of detecting ETM labels taught by CMS. The artisan would be motivated to substitute one known method of labeling (radioactive) for another method of labeling nucleotides (ETM). The artisan would be motivated to substitute the ETM label of CMS for the radioactive labels of Calzone, because the ETM labels eliminate the use of radioactivity and the hazards associated with the risk and because CMS teaches the ETM allows amplification of the signal and sensitive detection of target sequences. The artisan would be motivated to substitute the nucleases of CMS for those of Calzone as the nucleases of Calzone are substitutes and CMS teaches they will work. Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made that the ETM as exemplified by metallocene taught by CMS have a different electrochemical characteristics when attached to a base in an oligonucleotide as the art of record

recognizes the ETM attached to an oligonucleotide that is hybridized allows flow through the π orbital electrons which is not possible if the ETM is not attached to a base. Further it is obvious over the prior art that the electrochemical characteristics are dependent on the distance of the ETM from the electrode and thus the length of the nucleic acid. The artisan would have a reasonable expectation of success as the art demonstrates the claimed limitations are properties of the metallocene taught by CMS. The artisan would have a reasonable expectation of success by the combination of Calzone, Kayyem, Meade and CMS as they are known methods of detecting nucleic acids and have been demonstrated to work.

Response to Arguments

The response traverses the instant rejection.

The response asserts the teachings of CMS do not teach the electrochemical characteristics depending whether the marker is attached to a nucleotide, incorporated into an oligonucleotide, and the length of oligonucleotide. These arguments have been thoroughly reviewed but are not considered persuasive as the combined teachings of CMS, Kayyem and Meade demonstrate these limitations are obvious. Further it is noted teachings of the art of record encompass the metallocene labels of the instant specification, thus these alleged differences appear to be inherent properties of the metallocene labels of nucleic acids absent secondary considerations.
Thus the claims are obvious over the teachings of CMS, Kayyem and Mead.

The response further asserts Calzone in figure 2 teaches the presence of the non-degraded probe in the presence or absence of the complementary sequence. The

response further asserts that the amounts of the non-degraded probe in lanes 1 and 2 of Calzone cannot be distinguished by eye. These arguments have been thoroughly reviewed but are not considered persuasive the response is arguing one of skill in the art could not determine the amount of the nucleic acid species. The instant claims are drawn to detection of the presence or absence. Calzone if figure 2 teaches the presence of the degraded probe is only found in the presence of the complementary sequences, thus in combination with CMS, Kayyem and Meade rendering this claim and limitation obvious.

The response continues by asserting, "as the Examiner states at page 4, lines 11-14 of the instant Office action, CMS teaches that ETMs allow amplification of signal resulting in sensitive assays (see CMS page 54, lines 7-50 (sic)) and this provides for extremely specific and sensitive probes, which may detect target sequences without removal of unhybridized probe, Applicants submit that the signal so amplified is the result of using multiple and/or pluralities of ETMs (CMS at page 54, lines 8-16) and is not due to the different electrochemical characteristics of different oligonucleotide probes labeled with electrochemically active markers as claimed in claim 1 and as disclosed in the specification in Examples 4 and 5, Table 4 and Figures 8 through 13." These arguments have been thoroughly reviewed but are not considered persuasive as CMS envisions the use of multiple EMT on a probe on page 54 and in figure 32. Thus these argument are not persuasive.

The response continues by asserting that the different electrochemical characteristics of the different oligonucleotide probes could have not been predicted by

one of skill in the art. These arguments have been thoroughly reviewed but are not considered persuasive first as they are arguments of counsel that have not been substantiated by evidence. Further the teachings of Meade, Kayyem and CMS demonstrate the electrochemical activity of ETM depends of the size the nucleic acid it is attached to as well as being attached to the nucleic acid. Thus these arguments are not persuasive.

The response continues by asserting one of skill in the art would not be motivated to combine the teachings of Calzone and CMS. These arguments have been thoroughly reviewed but are not considered persuasive as the rejection states, "The artisan would be motivated to substitute the ETM label of CMS for the radioactive labels of Calzone, because the ETM labels eliminate the use of radioactivity and the hazards associated with the risk and because CMS teaches the ETM allows amplification of the signal and sensitive detection of target sequences. The artisan would be motivated to substitute the nucleases of CMS for those of Calzone as the nucleases of Calzone are substitutes and CMS teaches they will work. "

The response then continues by directing the examiner to the alleged unexpected properties of metallocene. These arguments have been thoroughly reviewed but are not considered persuasive as one of skill in the art based on the teachings of Kayyem that metallocene promotes electron transport through the π orbitals of nucleic acids, that metallocene not attached to a nucleic acid through a nucleotide would have different activities then a free metallocene. Further the teachings of CMS demonstrate the length of the nucleic acid the metallocene is attached

determines the electrochemical activity of the probe. Thus these arguments are not persuasive.

16. Claims 93 is rejected under 35 U.S.C. 103(a) as being unpatentable over Calzone et al (Methods in Enzymology (1987) volume 152, pages 611-632), Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001), Kayyem et al (US patent 6,096, 273 issued August 1, 2000) and Meade et al (WO 95/15971, published June 15, 1995) as applied to claims 1-9, 11-18, 20-25, 43-45, 91-101, 109-116, 119-129 above, and further in view of Nikiforov et al (US Patent issued May 21, 1996).

The teachings of Calzone, CMS, Kayyem, and Meade are set forth above.

Calzone, CMS, Kayyem, and Meade do not teach the use T7 exonuclease.

However, Nikiforov et al teaches the use of T7 exonuclease in primer extension assays to generate single stranded nucleic acids (see abstract). Nikiforov et al teaches that T7 exonuclease has the advantage over other nucleases that it has maximal activity in buffers suitable for DNA polymerase activity.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to improve the Calzone, CMS, Kayyem, and Meade electrochemical Invader based nucleic acid detection method by use of the T7 exonuclease taught by Nikiforov, because Nikiforov teaches T7 exonuclease can be used in the same buffer that amplification. The artisan would have a reasonable expectation of success as the artisan is substituting one known and characterized nuclease for another.

Response to Arguments

The response reiterates the arguments to the combination of Calzone and CMS and asserts the Nikiforov does not correct these deficiencies. These arguments have been thoroughly reviewed but are not considered persuasive as the arguments to Calzone and CMS have been addressed above and there are no specific arguments directed to the teachings of Nikiforov.

17. Claims 102-105 and 130-133 are rejected under 35 U.S.C. 103(a) as being unpatentable over Calzone et al (Methods in Enzymology (1987) volume 152, pages 611-632), Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001), Kayyem et al (US patent 6,096, 273 issued August 1, 2000) and Meade et al (WO 95/15971, published June 15, 1995) as applied to claims 1-9, 11-18, 20-25, 43-45, 91-101, 109-116, 119-129 above, and further in view of Heller et al (US Patent 5,605,662, filed Issued February 25, 1997).

Clinical Micro Sensors is here after referred to as CMS.

The teachings of Calzone, CMS, Kayyem, and Meade are set forth above.

Calzone, CMS, Kayyem, and Meade do not teach the use of electrochemical technique utilizing selectively one or more electrodes functionally surrounded by permeable membrane that is permeable on the basis of charge, size, or hydrophobicity.

However, Heller et al teaches the use of permeation layers covering electrodes that allow solvent movement, while allowing exclusion based on size and charge (see column 11, lines 3-23; column 13, lines 30-55). Heller teaches the use of charge in the permeability layer he also inherently teaches the use of hydrophilic layers, as charge molecules are hydrophilic. Heller teaches the permeation layer functionally

surrounding the electrode inhibits large proteins in the sample from binding the electrode, thus allowing the use of DC current (see column 11, lines 17-35). If the large proteins bound to the electrode, the large proteins would act as insulators, and cause a short circuit.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of Calzone, CMS, Kayyem, and Meade to include the use of a electrode with a selectively permeable membrane (permeation layer) of Heller, because Heller teaches the permeation layer allows the use of direct current without the insulating effects of large proteins binding to the electrode. The use of Heller's permeation layer would thus result in more accurate and sensitive assays. The artisan would have a reasonable expectation of success as the artisan is replacing one electrode used for the detection of nucleic acid with another electrode that has also been used to detect nucleic acids.

Response to Arguments

The response reiterates the arguments to the combination of Calzone and CMS and asserts the Heller does not correct these deficiencies. These arguments have been thoroughly reviewed but are not considered persuasive as the arguments to Calzone and CMS have been addressed above and there are no specific arguments directed to the teachings of Heller.

18. Claim 19, 117, 118 are rejected under 35 U.S.C. 103(a) as being unpatentable over Calzone et al (Methods in Enzymology (1987) volume 152, pages 611-632), Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001), Kayyem et al

(US patent 6,096, 273 issued August 1, 2000) and Meade et al (WO 95/15971, published June 15, 1995) as applied to claims 1-9, 11-18, 20-25, 43-45, 91-101, 109-116, 119-129 above, and further in view of Hall et al (US Patent 5,994,069 issued November 30, 1999).

Clinical Micro Sensors is here after referred to as CMS.

The teachings of Calzone, CMS, Kayyem, and Meade are set above.

Calzone, CMS, Kayyem, and Meade do not teach the use of a second recognition cassette that is labeled for detection of the cleavage reaction of a first partially hybridized complex.

However, Hall et al teaches a method of signal amplification using an invader probe, an unlabeled 1st probe, and a labeled second probe (see figure 96, and column 71 lines 45-52). Hall teaches the invader probe binds a first target sequence, while the 1st probe partially hybridizes the target and the unhybridized 5' tail of the 1st probe is released. The 5' tail of the 1st probe than hybridize a second target sequence, causing the 5' tail of the 2nd labeled probe to only partially hybridize to the second target sequence. Hall teaches the labeled 5' end of the second sequence is thus cleaved, released, and detected. The 2nd labeled probe and 2nd target are a recognition cassette.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improvement of the ETM invader method of Calzone, CMS, Kayyem, and Meade by use of Halls 2nd labeled probe and target. The ordinary artisan would be motivated to improve CMS method by use of Hall's 2nd labeled

probe and target, because Hall teaches it amplifies the signal, resulting in a more sensitive assay. The combined teachings of Calzone, CMS, Kayyem, Meade, and Hall would result in a more sensitive electrochemical invader assay, than that taught by CMS. The artisan would have reasonable expectation of success as the artisan is substituting one known method of nucleic acid detection for another.

Summary

No claims are allowed over prior art cited.

Conclusions

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN C. POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:30-4:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Steven C Pohnert/
Primary Examiner, Art Unit 1634